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Research Note

Salmonella Genotype Diversity in Nonlactating and Lactating Dairy Cows†

MICHAEL E. HUME,[1]* THOMAS S. EDRINGTON,[1] MIKE L. LOOPER,[2] TODD R. CALLAWAY,[1] KENNETH J. GENOVESE,[1] AND DAVID J. NISBET[1]

[1]U.S. Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit, 2881 F & B Road, College Station, Texas 77845; and [2]U.S. Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, Dale Bumper's Small Farm Research Center, Booneville, Arkansas, USA

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ABSTRACT

Dairy cows may serve as asymptomatic carriers of *Salmonella.* The potential for herd carrier status increases with herd size, and *Salmonella* shedding may be triggered by stresses placed on the animals. The scope of the current study is to determine the effects lactation may have on *Salmonella* genotypic diversity among detected serotypes. Fecal samples were collected on two sampling dates from 60 nonlactating and 60 lactating Holstein cows. No serotype was predominant over the two collection dates, although *Salmonella* Albany, *Salmonella* Anatum, *Salmonella* Newport, and *Salmonella* Senftenberg were detected in relatively high numbers. Twenty-three genotypes were detected on the first date and 27 on the second date. The greatest genotypic diversity was seen among *Salmonella* Newport and *Salmonella* Senftenberg, with five and nine genotypes, respectively. The presence of multiple serotypes and genotypes in the herd suggests multiple contamination sources. However, there was no conclusive effect of lactation status of the cows on *Salmonella* genotypic shedding.

Dairy cattle are important agricultural commodities in that they are a source of dietary milk and a growing source of nonfed beef. As with other food animals, dairy cattle may harbor bacteria of little concern to the health of the animal but of great potential threat to the well-being of human consumers. Milk pasteurization has minimized the threat associated with *Salmonella* contamination in raw milk *(19)*. However, with the growing dependence on cull dairy cattle as a source of beef, there is the increased potential for *Salmonella* contamination to enter the food chain *(7, 20)*.

Contaminating sources for dairy cows can range from feed, water, environmental factors, and handlers to new animals introduced into the herd *(5, 8, 10–13, 16, 17, 21, 24)*. Herd size can be used as an indicator for *Salmonella* contamination *(12, 22)*, and shedding status may be influenced by stresses that result from variables such as crowding, weather, lactation, and improper rationing *(5, 8, 11, 24)*. Lactation as a stress factor places a metabolic burden on the animal that results in heat generation *(24)*, which may be compounded by ambient temperature and humidity. The current study was conducted in conjunction with a larger study to determine *Salmonella* serotype distribution in nonlactating and lactating dairy cows. The scope of the current study is to determine the potential effects of lactation status on *Salmonella* genotypes found among serotypes in fecal samples.

* Author for correspondence. Tel: 979-260-9404. Fax: 979-260-9332. E-mail: hume@ffsru.tamu.edu.

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MATERIALS AND METHODS

Fecal samples and bacterial culture. Fecal samples (30 g) were collected by rectal palpation from 60 lactating and 60 nonlactating Holstein cows and placed in sterile containers and on ice for shipment to the laboratory. Samples were collected in August at 7:00 a.m. during the relatively cool portion of the day and at 5:00 p.m. *Salmonella* was cultured and serotyped according to the methods of Fitzgerald et al. *(6).* Sampled animals were part of a 3,000-head commercial dairy herd located in the southwestern United States. Cows averaged 4.3 years of age, were maintained in open pens with covered feed stands, and were restrained in self-locking stanchions for sampling.

PFGE. A single colony from each brilliant green agar plate incubated overnight at 37°C was placed in 10 ml of tryptic soy broth for overnight incubation and prepared in plugs for pulsed-field gel electrophoresis (PFGE) by the method of Hume et al. *(9)*. A quarter of each plug was incubated with *Xba*I restriction endonucleases according to the instructions of the manufacturer (New England BioLabs, Beverly, Mass.). Conditions for PFGE were as follows: initial switch time, 0.1 s; final switch time, 90 s; included angle, 120°; 6 V/cm; buffer temperature, 12°C; and run time, 22 h. Genotypic relatedness and dendrogram analysis were determined with Molecular Analysis Fingerprinting Software, version 1.6 (Bio-Rad Laboratories, Hercules, Calif.) using the Dice similarity coefficient and the unweighted pair group method using arithmetic averages for clustering.

RESULTS AND DISCUSSION

Twenty-three genotypes were found from the first sampling date among the 64 *Salmonella* isolates, representing

TABLE 1. *Genotypes of* Salmonella *fecal isolates from nonlactating and lactating dairy cattle*

	7:00 a.m.[b]		5:00 p.m.[b]		
Serotype[a]	D	L	D	L	Genotypes[c]
First sampling date					
Albany (6)	—	—	A (1) B (2) C (1)	C (1) D (1)	4
Dublin (3)	A (1)	A (1)	—	A (1)	1
Give (13)	—	A (7)	A (4)	A (2)	1
Havana (4)	—	A (1)	—	A (3)	1
Kentucky (1)	—	—	—	A (1)	1
Mbandaka (2)	—	—	A (2)	—	1
Meleagridis (7)	A (1) B (2)	B (1)	B (1)	B (1) C (1)	3
Newport (14)	—	A (2) B (1) C (1)	A (1) B (3) D (1)	B (3) D (1) E (1)	5
Senftenberg (8)	A (1) —	B (2)	A (1) D (2)	C (1) D (1)	4
Tennessee (6)		—	A (1) B (5)	—	2
Second sampling date					
Algona (1)	—	A (1)	—	—	1
Anatum (25)	A (2) B (3) D (6)	B (8) C (1)	D (3)	B (1)	4
Barranquilla (2)	A (1)	A (1)	—	—	1
Cubana (1)	—	A (1)	—	—	1
Dublin (1)	—	—	B (1)	—	1
Give (1)	—	A (1)	—	—	1
Kentucky (1)	—	—	B (1)	—	1
Mbandaka (2)	—	—	—	B (1) C (1)	2
Meleagridis (6)	D (3)	—	A (1) D (1)	D (1)	2
Montevideo (1)	—	A (1)	—	—	1
Newport (8)	A (2) B (1)	A (2)	A (1) C (1)	A (1)	3
Senftenberg (14)	E (1) F (2) G (1) H (1) I (1)	C (1) F (3) G (1)	C (1) J (2)	F (1) J (2)	7
Untypeable (1)	—	A (1)	—	—	1
6,8:1,2-monophasic (1)	—	—	—	A (1)	1

[a] The total number of isolates for each serotype is given in parentheses.

[b] D, nonlactating; L, lactating. Letters are arbitrary designations for genotypes within each serotype. Values in parentheses indicate the number of isolates with a shared genotype.

[c] The total number of genotypes for each serotype.

10 serotypes in nonlactating and lactating dairy cows (Table 1). The greatest genotypic diversity loosely corresponded to those serotypes found in greatest abundance (i.e., *Salmonella* Albany, *Salmonella* Newport, and *Salmonella* Senftenberg), with exceptions being *Salmonella* Give, *Salmonella* Meleagridis, and *Salmonella* Tennessee. *Salmonella* Give accounted for roughly 20% of the total number of isolates detected on the first sampling date, but its single genotype amounted to only approximately 4% of the 23 genotypes identified. *Salmonella* Newport exhibited the greatest genotypic diversity, with its five genotypes representing approximately 22% of the genotypes identified. *Salmonella* Albany and *Salmonella* Senftenberg were slightly less diverse, with approximately 17% each of the total number of genotypes identified, followed by *Salmonella* Meleagridis and *Salmonella* Tennessee. A dendrogram and macrorestriction patterns for *Salmonella* Senftenberg are shown in Figure 1.

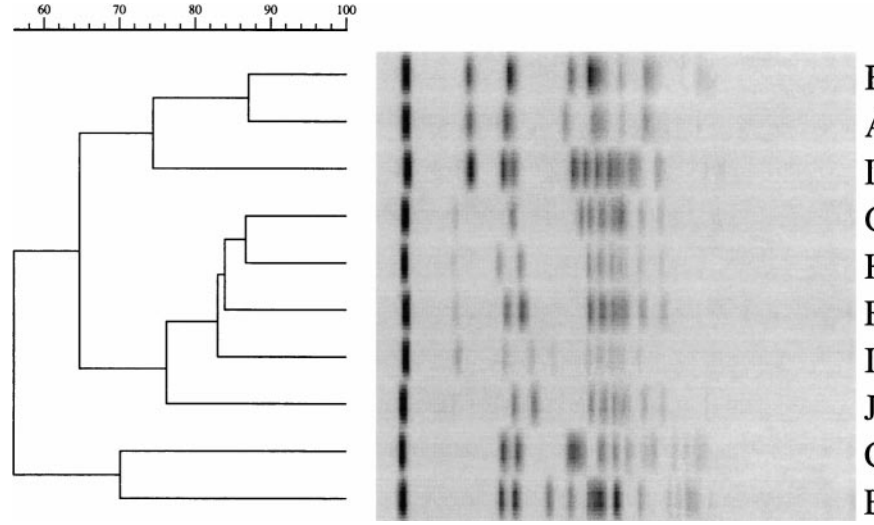

FIGURE 1. *Dendrogram and pulsed-field gel electrophoresis profile of* XbaI *genotypes of* Salmonella *Senftenberg isolates from nonlactating and lactating dairy cows. Letters are arbitrary indicators of genotype. The bar above the figure indicates percentage of similarity coefficient.*

There were 27 genotypes among the 65 isolates and 14 serotypes from the second sampling date (Table 1). Although *Salmonella* Anatum was the isolate most often found, at approximately 42% of all isolates, it accounted for only four or approximately 15% of the genotypes. The greatest diversity in this sampling was found among *Salmonella* Senftenberg isolates at approximately 26% of genotypes. Three (genotypes A, B, and C) of the four *Salmonella* Senftenberg genotypes detected in the first sampling were present in the second sampling, whereas nine of the 10 total *Salmonella* Senftenberg genotypes in the study were present in the second sampling. *Salmonella* Newport (11%) was next in the diversity ranking followed by *Salmonella* Mbandaka and *Salmonella* Meleagridis, with approximately 5% each of the genotypes detected.

Those serotypes represented by relatively large numbers of isolates, but only one or a few genotypes, may have been introduced into the herd from one or a few sources. Accordingly, other genotypes represented by a correspondingly high number of serotypes (e.g., *Salmonella* Newport and *Salmonella* Senftenberg) may have come from several contaminating sources. The failure to detect specific genotypes or serotypes on either collection date may not be indicative of the absence of those isolates from the herd. A negative status may be attributed to the animal tested being entirely clear of *Salmonella* or clear of a particular genotype or serotype or the animal may be exhibiting intermittent shedding. Intermittent shedding of enteropathogens by food animals is a common phenomenon *(1, 9, 18, 23)*, but factors associated with intermittent shedding are still unknown.

Even though none of the genotypes detected among serotypes (excluding those isolates that share identical genotypes and several Meleagridis isolates with more than 93% similarity) were closely related (more than 90% similarity), after plain visual comparison, some of the isolates shared multiple macrorestriction bands of the same sizes (e.g., *Salmonella* Senftenberg genotypes F, G, H, and I). The sharing of macrorestriction bands may be explained in terms of genomic rearrangement and recombination and would contribute to the relatively high level of genotypic diversity seen in some serotypes *(2–4, 14, 15)*. A tendency toward genomic plasticity is thought to confer adaptive advantage in hostile environments *(3)*. Similar to phenomena seen in some *Campylobacter* isolates, some *Salmonella* serotypes or strains may exhibit a high degree of genotypic variability. Frequent expressions of genomic variability may be caused by spontaneous rearrangement of intragenomic segments, as well as intergenomic recombination that results from new DNA segments being introduced from external sources.

In conclusion, findings from the current study indicated no conclusive differences in *Salmonella* genotypes among nonlactating and lactating dairy cows from the same herd. The genotypic diversity seen with some serotypes may be indicative of multiple contaminating sources; however, genomic factors related to sequence heterogenicity may add to the variability exhibited by some serotypes.

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